Gene Editing and its Presence in Healthcare

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What seemed like science fiction not long ago is now becoming a reality: scientists are editing human DNA. Though it hasn't been around for long, using gene editing to treat diseases or enhance certain favorable traits has proven to be promising. While the idea is exciting to most, the possibility of such a powerful tool has also sparked controversy in multiple arenas. This paper will explore the prominent developments in gene editing and gene therapy, its progress in healthcare, and the ethical debates that arise as a result.

Background to DNA, Genetics, and Mutations

As first discovered by Roslaind Franklin, DNA has a double helical structure, and each helix has nucleotides that pairs up with specific nucleotides on the other helix. There are four nucleotides that pair up with each other: Adenine, which pairs with Thymine, and Cytosine which pairs with Guanine. The DNA sequence refers to the order of these nucleotides on the DNA molecule.

Genes are small segments of the gnome that code how to make proteins. Alleles are copies for a specific gene. Each gene is usually coded by two alleles, one from each parent, and they can be homozygous, meaning both alleles are the same type, or heterozygous, meaning each one is different. Alleles are also commonly described as either dominant or recessive. Dominant alleles dominate the expression of the gene, overpowering the recessive gene even if it's present. Recessive alleles, however, can only be expressed without the presence of the dominant gene. With diseases, if the condition is autosomal dominant, they are affected with the disease if they have even one of the dominant alleles. If it is autosomal recessive, people can be carriers of the disease if they have only one recessive allele.

Sometimes, genetic mutations that change a DNA sequence can occur, and sometimes, the protein affected by the mutation, or the lack of it, can cause disease. There are four main types of mutations: Silent, substitution, insertion, and deletion mutations. Silent mutations ultimately do not affect the protein, because even though the sequence has been changed, it still codes for the same protein. Substitution mutations exchange one codon for another, which is a small change but can have a big impact on the protein produced depending on the context. In an insertion mutation, an entirely new base pair is added in addition to the ones already there, and a deletion mutation removes a base from the sequence entirely. Both of these mutations can cause a frameshift, moving the entire sequence over which could possibly code for an incorrect protein or a stop codon that would stop the rest of the sequence from being read.

Introduction to Gene Editing and Gene Editing Tools

Humans have been manipulating genetics long before they started touching human gene editors. To name a few major points, selective breeding between animals and plants has been around for a very long time. In 1973, scientists introduced an antibiotic resistance gene into a bacteria strain, and in 1992, the tomato was genetically modified to have more commercially desirable qualities; a practice that was closely followed with several other foods.

When DNA is broken or in need of repairs from mutations or other issues, the human body has natural mechanisms to repair itself. The two main processes the body uses to repair double stranded breaks are non-homologous end joining and homology repair. In non-homologous end joining, the ends of the strand on either side of the break are simply joined

together. This process is what is most frequently used because when there is a break, the body attempts to repair it as soon as possible. However, it is very error prone and can introduce a lot of different issues because it is so quick and messy. Homology repair uses a template of the DNA strand to make precise repairs on that strand. Since it requires so many different protein elements to fix it, it is not as frequently used.

Some of the classic gene editing techniques that first appeared in the field include meganuclease, Zinc Fingers, and TALENs. Meganuclease creates a double stranded break in the DNA sequence where the mutation is. Though it does recognize some relatively big sequences in the DNA it has several off-target effects and has a limited range of applications. The Zinc FInger technique refers to the zinc-finger domains that scientists found and were able to bind certain FokI restriction endonucleases to. These endonucleases could bind to the DNA based on the specific finger domains and cut the DNA at those sights. This method is very programmable, but didn't take off because it's very expensive and difficult to work with. TALENs are DNA binding domains that were taken from plant bacteria originally and are a result of protein engineering. There are more domains to interchange to target different areas in gnome with TALENs than with zinc fingers. They are also overall cheaper and more flexible to design, but they are hard to clone.

Another group of gene editors involve the Cas9 enzyme. CRISPR Cas9 uses CRISPR arrays, or memories, created when viruses attack. In this technique, a guide RNA will be created to direct a certain CRISPR array with the Cas9 enzyme to the invading DNA injected. Cas9 will approach and create a double stranded break to prevent it from being used and replicated (natural repair mechanisms will take control after this). This technique is mostly used for gene knockout, but has a variety of uses. CRISPR Cas9 is well-known because it is precise, cheap, and highly programmable. However it has a higher potential for off target effects because of the range of where the guide RNA could bind. It also created a double stranded break, which could always cause unknown or unintended consequences. Base editing is a newer method that uses a catalytically dead Cas9 enzyme. The enzyme can still target DNA, but just binds to it instead of cutting it, after which a domain called Cytidine Deaminase fuses with other domains to chemically alter the structure of the base pairs to change it to a different base. No breaks are created here and it's precise, however, it's really only applicable in really specific disease scenarios because it only edits one base pair. Prime editing uses Cas9 to bind to a target DNA using a guide RNA and cut only one strand of it. This single strand is primed by a pegRNA site and leaves the edit hanging off of the prime editor. The other DNA in the body matches up to this strand which acts as a template and uses several different repair systems to make the strand match up.

Gene Therapy Trials

Most gene therapies are packaged in viruses, generally adenovirus, AAV, retrovirus, and lentivirus. These viruses have different carrying capacities, affect different cells, and have different safety levels.

Gene therapies are administered in an ex vivo or in vivo way. With ex vivo, cells are removed from patients, cultured, infected with the therapeutic gene, and then administered back into the person. In vivo therapies are administered directly into the person through viruses. In all scenarios, clinical trials are required to test safety, effectiveness, value, and relativity.

One of the first diseases to be treated with gene therapy was ADA-SCID. It's a rare disease that results in the patient having no immune protection and having to be heavily

sheltered. Treatments can be done, for example by injecting an artificial ADA enzyme called PegADA, however the effectiveness of this treatment wanes over time and a patient can stop responding altogether at one point. This was the case with Ashanti Desilva, an ADA-SCID patient who was later chosen for a gene therapy trial. She was injected with a retrovirus containing a working copy of the ADA gene. She got small does every two months from then on, but it's theorized that she might not have needed them at all. The trial was very successful, as she almost fully recovered most functions.

Progress has also been made with using gene therapy to treat Duchenne's Muscular Dystrophy, or DMD. DMD is characterized by weakened muscle tissue and caused by a mutation in the dystrophin gene. However, since this gene is one of the largest in the body, it's hard to fit the gene into a virus. So, researchers created a microdystrophin gene by splicing different expressions together and administered it through AAV. When administered on golden retriever dogs with DMD, the effects were very promising. A prominent case of DMD being treated in humans is the trial with Conner Curran, a young boy with DMD. His treatment also used the AAV virus with Pfizer to administer a microdystrophin expression. He showed vast improvements in a relatively short amount of time, and he was one of the several patients who showed these vast improvements. Though there were some immune reactions, none were very severe, and the treatment allows him to function relatively normally with a less severe version of muscular dystrophy overall.

Some cases didn't turn out to be successful, as was the case with Jesse Gelsinger, a teenager with a relatively mild form of OTCD. OTCD is an x-linked disorder characterized by a mutation that disrupts the Urea cycle. This disruption means that the body can't process ammonia properly, leading to a buildup of ammonia in the blood which can lead to liver damage. Jesse participated in an OTCD gene therapy trial with the main motivation of helping people who had more severe forms of his condition. After he was injected with the virus containing a functioning OTC gene, his immune system had a severe response, which led to a rapid decline in his health, and led to eventual death. It was later discovered that there were several problems with the trial, including the eligibility requirements that Jesse never should have fit in since his liver enzyme levels were high enough to exclude him from the trial completely. The risks of the trial weren't communicated to Jesse or his family clearly, and it's unclear whether the previous results of the treatment were properly shared with them or not. Overall, this case shook the gene therapy community and put the field on pause for a while.

Gene Editing Trials

Gene editing varies from gene therapy in that instead of administering a working copy of the gene into the body, the genes themselves are edited.

One of the most promising cases in the gene editing field is the progress with Sickle Cell Disease. This is an autosomal recessive disorder in which patients have mutations in the hemoglobin gene, causing their blood cells to be sickle shaped which can lead to several complications. It has few treatment options, with patients often having events of severe pain. In the body, there are multiple forms of hemoglobin, A (adult) hemoglobin and F (fetal) hemoglobin. When someone is born their body produces F hemoglobin, but this gets naturally repressed by the BC11A part of the gnome as they get older, at which point A hemoglobin starts being produced in greater and greater quantities until F hemoglobin stops being produced at all. With this treatment, instead of editing the A hemoglobin gene, researchers targeted the BC11A, which acted as an off switch for F hemoglobin, so that the F hemoglobin could be turned on. In

the case of Victoria Gray, this treatment worked very well. She underwent an ex vivo modification of BC11A in CD34+ cells following myeloablation, which means eliminating several parts of the immune system to create places for the new T-cells to reside. The immune system was weakened beforehand as well to avoid severe reactions. After the treatment, Victoria's VOCs (the painful events) had essentially vanished, and her HbF levels (fetal hemoglobin levels) increased drastically as well. She only needed one treatment for a long time period, and needed very few additional transfusions. Based on this progress and several other developments, it's anticipated that the first approved gene editing therapy might be for Sickle Cell Disease.

While the gene editing developments for SCD have been successful, few others have shown such progress. Huntington;s ideas, for example, is something that hasn't shown enough potential to be in the clinic for anything gene editing related. This disorder is autosomal dominant, very rare, but very severe. It's a progressive brain disorder that eventually leads to death. It's caused by a trinucleotide CAG code repeat expansion. CAG codes for a certain amino acid, and usually there are only ten to twenty-six of those repeats. In Huntington's disease, patients often have thirty seven to ninety of them, which leads to a long and jumbled RNA strand that wasn't intended. To test the treatment, scientists first created a live model of Huntington's disease in pigs by using Cas9 to splice additional CAG repeats into a normal pig. The actual treatment used in vivo therapy to insert CRISPR Cas9 directly into the brain that aimed to splice some of the CAG repeats. The pigs they treated showed less production of the HTT protein and were able to walk in a straighter line than the untreated pigs. The process, however, took very long, about 5 years, and didn't show results that were extremely promising. Most of the effects were off-target insertions and deletions that weren't intended, since Cas9 creates double stranded breaks. Overall, while the pigs lived a bit longer, the treatment didn't create a big impact.

Another in vivo gene editing clinical trial was with LCA10 which is a relatively rare autosomal recessive disease caused by a mutation in the SEP290 gene. People with this condition have severe and early vision detriments because their pupils don't react the same way to light. The mutation is an intron, which CRISPR Cas9 targets to cut it out and allow the exons to splice together properly. It's packaged in an AAV and injected directly into the retinal cells in the back of the eye. The trial made it to the second phase, and out of the 14 patients treated, 3 showed improvements. Two of the patients that did show improvements, it was later shown, were homozygous for the mutated gene. Therefore, it's likely that the gene editing was more effective for them because both of the genes were edited, producing more of the SEP290 protein. While these patients did show vast improvements, there were still eleven patients who didn't show any results. There hasn't been much attention on the treatment since then since on top of being very expensive, it would only be helpful to a very small portion of an already rare disease.

Considerations of Gene Editing in Human Health and Disease

With the progression of gene editing and therapy treatments, and the possibility that they will soon be available to a wider population comes the ethical and health concerns of these treatments.

Some of these health concerns include off-target effects, cancer, mosaicism, and immune reactions. Off-target effects are the main effect that can be expected from these treatments, and the severity of these effects can vary. Depending on how the specific treatment is, these off

target effects could be something as big as creating a brand new phenotype. Some off-target effects, or the editing of the wrong gene could result in cancer, as it could lead to uncontrolled cell division and the growth of tumors. Mosaicism refers to different edits in different cells that are in separate areas. Sometimes, these traits are visible, for example in multicolored cats, in which different genes are expressed in different areas of the cat's hair. Chimera is when 2 different embryos are fused, which can happen if only one group of cells are edited, and they fuse with the unedited cells. This can occur naturally in humans as well, for example, people who have two different colored eyes, or a different colored streak in their hair. Immune reactions are also a very important effect to consider, as any time anything foreign is put into the body, the immune system reacting to it is always a concern. Since CRISPR Cas9 is isolated from bacterial systems, the body will probably already have antibodies against these viruses, especially if the patient has already had them. This could have implications if they are trying to receive the therapy, as their immune system might react not only to the virus package, but also to the actual Cas 9 editor. An example of a fatal immune reaction is the Terry Hogan case. Terry had DMD but wasn't accepted into any of the clinical trials for it because he had some other complications that posed several risks. His brother then founded a non-profit so that Terry could get the treatment, and he did, however after a dose of the therapy has a terrible immune reaction to it and passed away.

The ethical considerations of gene edits and therapies vary per person as they can be influenced by culture, life experiences, and worldview in general. The general guidelines however are led by the Principles of Bioethics as proposed by Beachamp and childress. These principles are autonomy, justice, beneficence, and non-maleficence.

The main questions surrounding the issue deal with how to prioritize diseases, how they should be regulated, and who should be the ones to decide all these things. One case that sparked these ethical concerns came in 2018, when a man named He Jiankui announced that he had edited some embryos to have a mutation on the CCR5 gene that would grant them immunity to HIV. There was a strong, negative reaction to this, first of all, because there are ways to have babies without HIV that are far less risky, and these babies were germline edited, meaning all these edits would be passed down to future generations. He Jiankui was eventually sent to jail and fined, leaving the gene editing field shaken. Another controversy came when the genetic cause of deafness was edited to treat the condition. A lot of the deaf community didn't appreciate this because many of them don't see deafness as a disease to begin with, and never asked for a treatment for their condition. Much of the community was upset because they weren't even brought in for discussion regarding the treatment. Both of these cases left the gene editing community with many questions about who should regulate these tools, how they should be used, and when they should be used.

Conclusion

Ultimately, gene therapies and edits have come a long way and are showing promising signs in the world of healthcare. As the field progresses, previous successes and mistakes are also being considered, as well as the ethics and health concerns that appear along the way.

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